Inosine Assay Kit

Cat. No. Kit-0477 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	The Inosine Assay Kit provides a simple and direct procedure for measuring inosine in a variety of samples. Inosine concentration is determined by a coupled enzyme reaction in which inosine is converted to hypoxanthine which reacts with the substrate mix and probe, resulting in a fluorometric product (Ex/Em = 535/587 nm), proportional to the inosine present.
Description	Inosine is a purine nucleotide found at the wobble position of tRNA where it plays a role in the proper translation of mRNA at the ribosome. In addition to its role in translation, inosine also plays important roles in the immune system where is exhibits both inflammatory and anti-inflammatory effects. Inosine may be tissue protective during ischemic injury and recent reports suggest inosine may preserve cell viability during hypoxia.
Storage	The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended.
Shipping	Gel Pack
Size	100 assays
Kit Components	Inosine Assay Buffer: 25 ml Inosine Probe: 0.4 ml Converter Enzyme Mix (lyophilized): 1 vial Developer Enzyme Mix (lyophilized): 1 vial

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	Inosine Substrate Mix (lyophilized): 1 vial Inosine Standard (10 mM): 50 µ
Materials Required but Not Supplied	· 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays. · Fluorescence multiwell plate reader · 60% Perchloric acid · Potassium carbonate · Sodium Hydroxide
Preparation	Briefly centrifuge vials before opening. To maintain reagent integrity, avoid repeated freeze/thaw cycles. Inosine Assay Buffer – Allow buffer to come to room temperature before use. Converter Enzyme, Developer Enzyme, Inosine Substrate Mix – Reconstitute each in 220 mL of Inosine Assay Buffer. Mix well by pipetting, then aliquot each and store, protected from light at –20 °C. Use within 2 months of reconstitution.
Assay Protocol	All samples and standards should be run in duplicate. Inosine Standards for Fluorometric Detection: Dilute 5 mL of the 10 mM (10 nmole/mL) Inosine Standard Solution with 995 mL of Inosine Assay Buffer to prepare a 50 mM (0.05 nmole/mL) standard solution. Add 0, 2, 4, 6, 8, and 10 mL of the 50 mM Inosine standard solution into a 96 well plate, generating 0 (blank), 0.1, 0.2, 0.3, 0.4, and 0.5 nmole/well standards. Add Inosine Assay Buffer to each well to bring the volume to 50 mL. Sample Preparation: Liquid samples like serum and plasma can be measured directly. Tissue (10–100 mg) or cells (5x10^6) can be homogenized in 0.7 mL of ice-cold 0.4 M perchloric acid. Centrifuge the samples at 13,000 x g for 10 minutes to remove insoluble material. Transfer the supernatant to a new tube and then neutralize by

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	adding 10 mL of 4 M potassium carbonate solution per 100 mL volume. Incubate on ice for 7–10 minutes. Centrifuge the neutralized supernatant at 13,000 x g for 10 minutes. Add samples to wells or freeze at –80 °C until ready to analyze. For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve. If desired, the pellet from the perchloric acid homogenization step can be dissolved in 300 mL of 0.5 M sodium hydroxide solution and used for total protein analysis. Bring samples to a final volume of 50 mL with Inosine Assay Buffer. Notes: Xanthine, hypoxanthine, and NADH in the samples can generate a background signal. To remove the effect of background, a sample blank may be set up for each sample by omitting the Converter Enzyme from the reaction mix. Assay Reaction: 1. Set up the Reaction Mixes according to the scheme in Table 1. 50 mL of the appropriate Reaction Mix is required for each reaction (well). Table 1. Reaction Mixes Reagent Samples and Standards Sample Blank Inosine Assay Buffer 40 mL 42 mL Inosine Probe 4 mL 4 mL Inosine Substrate Mix 2 mL 2 mL Converter Mix 2 mL – Developer Mix 2 mL 2 mL 2. Add 50 mL of the appropriate Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 30 minutes at 37 °C. Cover the plate and protect from light during the incubation. 3. Measure fluorescence intensity (Ex/Em = 535/587 nm).
Analysis	Calculations: The background for the assays is the value obtained for the 0 (blank) Inosine Standard. Correct for the background by subtracting the 0 (blank) value from all

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readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate Inosine standards to plot a standard curve.
Note: A new standard curve must be set up each time the assay is run.
Subtract the sample blank value from the sample reading to obtain the corrected measurement. Using the corrected measurement, the amount of Inosine present in the samples may be determined from the standard curve.

Concentration of Inosine:
Sa/Sv = C
Sa = Amount of Inosine in unknown sample (nmole) from standard curve
Sv = Sample volume (mL) added into the wells
C = Concentration of Inosine in sample
Inosine molecular weight: 268.23 g/mole

Sample Calculation:
Amount of Inosine (Sa) = 0.58 nmole (from standard curve)
Sample volume (Sv) = 50 mL
Concentration of Inosine in sample
0.58 nmole/50 mL = 0.0116 nmole/mL
0.1168 nmole/mL x 268.23 ng/nmole= 3.11 ng/mL

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